United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

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Title: Procedure for Testing and Maintenance of Control Strains		
Revision: Revision 1	Replaces: 05/15/04	Effective: 08/01/04

1. Purpose:

To provide standard procedures for the testing and maintenance of all control strains.

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for the USDA/AMS Microbiological Data Program (MDP), including support laboratories conducting non-routine activities that may impact the program.

3. Principle:

All MDP laboratories will use the control strains specified in each method SOP. Strains are preserved as **master stock cultures** for maintenance and **working stock cultures** for testing and distribution. The AMS National Science Laboratory (NSL) will perform appropriate tests on control strains and parent strains of the control strains, when necessary. These tests include: identification of the strain by VITEK, growth on selective agar media, testing by BAX Polymerase Chain Reaction (PCR) and any antigen-antibody reaction-based method, and expression of *gfp* by exposure to UV light at 365 nm. NSL will also distribute strains to the participating laboratories. If problems are encountered with any control strain, MPO shall be notified. NSL will ship a new stock to replace the problem stock.

The positive control strains include those carrying *gfp* on the chromosome. Successful recovery of the control strains (as opposed to naturally-contaminating microflora) from the analytical procedures can be determined by specific biochemical and immunological tests, as listed above.

4. Outline:

5. References:

5.1 Bacteriological Analytical Manual Online, Food and Drug Administration; http://www.cfsan.fda.gov/~ebam/bam-toc.html (last accessed 3-2-04)

5.2 Monday, S.R., S.D. Weagant, and P.Feng. 2003. Use of endogenous host plasmids for generation of *Escherichia coli* O157:H7 and *Shigella sonnei* that stably express the green fluorescent protein. Plasmid. 50: 161-167.

5.3 Barak, J.D., L.C. Whitehand, and A.O. Charkowski. 2002. Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. Appl. Environ. Microbiol. 68: 4758-4763.

5.4 Miller, W. G, J.H. Leveau and S.E. Lindow. 2000. Improved *gfp* and *inaZ* broad-host-range promoter-probe vectors. Mol. Plant-Microbe Interact. 13: 1243-1250.

5.5 SOP MDP-LABOP-02, Sample Receipt and Elution Procedure

5.6 SOP MDP-SHIP-03, Packaging, Shipping, and Archiving of MDP Cultures

5.7 SOP MDP-QA-03, Quality Assurance (QA) Controls

6. Specific Procedures:

This SOP represents minimum MDP requirements and is presented as a general procedure. Each laboratory shall have written operating procedures that provide specific details concerning the manner in which the procedures have been implemented in that laboratory, as deemed necessary by the laboratory for ISO or other quality control (QC) certification.

6.1 Equipment and Materials

6.1.1 Positive and negative control bacterial strains (see SOP MDP-QA-03 for details)

6.1.1.1 **MDP-001** *E. coli* O137:H41 Strain MW 421 (USDA/ARS/PW #RM2375) (transformed #RM3658), a cabbage root isolate (working designation: *E.coli* #3658 pKT-kan.) CDHS/MDL #00A 3563 (USDA/ARS/PW #RM2375)

6.1.1.2 **MDP-002** *Salmonella enterica* serovar Poona (working designation: *S. poona* #2350 pKT-kan.), a human clinical isolate from an outbreak associated with cantaloupe

6.1.1.3 **MDP-003** *Enterobacter aerogenes* (ATCC strains)

6.1.1.4 **MDP-004** *E. coli* O157:H7 (ATCC 43890-GFP), constructed in the laboratory of Peter Feng, FDA

6.1.1.5 **MDP-005** *E. coli* (CGSC# 7923; Δ*(argF-lac)169*, Δ*uidA3::pir*$^{+}$, *rpoS396(Am)?, rph-1, hsdR514*)

6.1.1.6 **MDP-013** *Shigella sonnei* (F2353-GFP), constructed in the laboratory of Peter Feng, FDA

6.1.1.7 **MDP-014** *Salmonella typhimurium* UK-1 (Δ*asd*/pYA3553-GFP3), constructed in the laboratory of Roy Curtiss, Washington University

6.1.2 Materials, media and reagents required for testing routine MDP produce samples.

6.1.2.1 Non-specific rich broth such as BHI, Nutrient broth

6.1.2.2 Nutrient agar plates

6.1.2.3 CHROMagar O157 agar plates

6.1.2.4 CHROMagar *E. coli* agar plates or similar types that contain MUG

6.1.2.5 EMB and/or MacConkey agar plates

6.1.2.6 BS, HE, and XLD plates for *Salmonella*

6.1.2.7 Congo Red agar for *Shigella*

6.1.2.8 Specific media for test samples: specific control cultures for positive produce controls should be added to the following media depending upon the method

6.1.2.8.1 LST-ColiComplete (MUG) for *E. coli* – refer to SOP MDP-MTH-01

6.1.2.8.2 Lactose broth for *Salmonella* – refer to SOP MDP-MTH-04

6.1.2.8.3 Modified EC broth + novobiocin for *E. coli* O157:H7 – refer to SOP MDP- MTH-05

6.1.2.8.4 *Shigella* broth (FDA-BAM) + novobiocin for *Shigella* spp. – refer to SOP MDP-MTH-08

6.1.2.9 Sterile inoculating loops, hockey-sticks, etc.

6.1.2.10 Incubator set at 35 ± 2°C

6.1.2.11 Sterile test tubes and flasks

6.1.2.12 Antibiotics kanamycin

6.1.2.13 Nutrient agar plates containing kanamycin (50 µg/mL)

6.1.2.14 Long wavelength UV light source

6.1.3 Stock solution: Kanamycin

Dissolve 100 mg Kanamycin monosulfate (Fisher Catalog# BP906-5, or Sigma Catalog# K1377, 5 grams or equivalent grade) or equivalent in 2 mL distilled water. Mix to dissolve, filter sterilize (0.2 µm syringe filter B-D # 309602 - Fisher Catalog # 09-719C or equivalent), and store at 4°C or frozen (-80°C) for long-term storage. Add 1 mL per liter to sterile culture medium (after cooling to ~ 50°C) for a 50 µg/mL final concentration.

6.2 Reviving and Growth of Strains (MDP Culture Repository Only)

Revive strains by streaking them on Nutrient agar plates followed by incubation for 24 hours at 35 ± 2°C. Visually check for purity.

6.2.1 For strains sent on discs (e.g., 1 cm diameter discs): Place the disc on a Nutrient agar plate. Wet the disc with a small volume (0.1 mL) of sterile solution (saline, Phosphate Buffered Saline, or any rich broth) using a sterile pipette or a micropipette with a sterile tip. Using a sterile loop, streak the solution from the disc across the agar surface for colony isolation. Invert the plate and incubate for 18-24 hours at 35 ± 2°C.

6.2.2 For strains sent in stab-vials: Using a sterile loop, scoop out cells from the stab and streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate for 18-24 hours at 35 ± 2°C.

6.2.3 For strains sent on slants: Scrape cells using a sterile loop and streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate for 18-24 hours at 35 ± 2°C.

6.2.4 For strains sent on MicroBank beads: Aseptically remove one bead using sterile needle or forceps. Immediately close the vial tightly and return it to the low temperature storage as soon as possible. Streak on a Nutrient agar plate or drop into a rich broth and incubate for 18-24 hours at 35 ± 2°C.

6.3 Maintenance and Storage of Cultures

6.3.1 Maintenance of the cultures

Control strains that contain genes coding for GFP and kanamycin resistance (kanR) integrated onto the chromosome should be grown on a non-specific rich media containing kanamycin. It is advisable to use the control strains that have the genes coding for GFP and kanR integrated onto the chromosome because these cultures can be grown for several generations without the selective pressure from antibiotics. In order to ensure that the plasmid is maintained or the genes are integrated onto the chromosome, growth of these

strains in the presence of antibiotic should be tested; however, do not add antibiotics to the positive produce control.

Strains that do not carry genes coding for GFP and kanR can be grown in the non-specific rich medium and inoculated into the specific pre-enrichment media (e.g. mEC+novobiocin for *E. coli* O157:H7, LST+ColiComplete [MUG] for *E. coli*) as positive, negative, or positive produce controls.

6.3.2 Storage of the cultures: For making master and working stocks of strains follow MicroBank beads manufacturer's directions as follows (refer to product insert for additional details):

6.3.2.1 To prepare stocks, open the cryovial screw cap under aseptic conditions. Inoculate the cryopreservation fluid with cells (use sterile loop) from 18-24 hour culture plates to a density of approximately three to four McFarland standard. Close vial and gently invert four to five times to suspend organisms. Do not vortex.

6.3.2.2 Remove screw cap and aspirate the excess cryopreservation fluid aseptically. Tighten the cryovials. Inoculate desired number of cryovials (minimum requirement: 1 vial as a master and 2 vials as working stocks).

6.3.2.3 Label with the name of the bacteria, date, passage number, and initials. Place in cryotube holder. Store at -70ºC.

6.4 Tests

6.4.1 For detection of control strains that carry genes coding for GFP in broth or on agar, the cultures can be exposed to long wavelength UV at 365 nm. These cultures will show green to bluish-green fluorescence, indicating the expression of GFP. At times, the intensity can be enhanced after holding the cultures at room temperature for 1-2 hours following routine incubation. For *E. coli* O157:H7-GFP cultures, addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) will enhance the fluorescence.

6.4.2 For other strains (i.e., those not carrying the gene coding for GFP), use selective agar media (e.g. EMB, MacConkey, CHROMagar, XLD, etc.) for distinguishing the control strains from the isolates; *E. coli* CGSC strains and *E. coli* O157 can be distinguished based on the lactose fermentation and MUG hydrolysis. In addition, CHROMagar O157 should be used to confirm *E. coli* O157.

6.4.3 Additional biochemical tests may also be required to distinguish control strains and will be listed in each method SOP.

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Revision 01 July 2004 MPO

- Eliminated tetracycline stock preparation instructions from subsection 6.1.3
- Deleted unused control strains, MDP-006 and MDP-007, from subsection 6.1.1